J. Biochem. 127, 591–596 (2000)

Effect of Human Granulocyte-Macrophage Colony Stimulating Factor (hGM-CSF) on Lymphoid and Myeloid Differentiation of Sorted Hematopoietic Stem Cells from hGM-CSF Receptor Gene Transgenic Mice[1]

Tadashi Okubo,* Nobuaki Yanai,* Sumiko Watanabe,† Ken-ichi Arai,† and Masuo Obinata*,[2]

*Department of Cell Biology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, 980-8575; and †Department of Molecular and Developmental Biology, Institute of Medical Sciences, The University of Tokyo, Tokyo

Received December 9, 1999; accepted January 19, 2000

Bone marrow lineage-negative (Lin^-) c-Kit^+ $Sca\text{-}1^+$ hematopoietic cells from human GM-CSF receptor gene transgenic mice were cultured on established bone marrow stromal cell (TBR59) layers and on semisolid medium. In the semisolid assay, an increasing number of larger colonies were observed in the presence of hGM-CSF. By coculture with the stromal cells, cobblestones containing myeloid and lymphoid lineages of cells were formed from the stem cell enriched fraction, and addition of hGM-CSF strongly stimulated formation of the cobblestones containing both lineages. Repeating passages of the cobblestones on TBR59 stromal cells in the presence of hGM-CSF gradually decreased cobblestone formation and inversely increased macrophages and granulocytes, while mast cells were generated when the cells derived from the semisolid assay were cultured in a liquid medium containing hGM-CSF. These results consistently suggest that cytokines such as GM-CSF may costimulate the immature hematopoietic cells at their stroma-dependent phase before lineage commitment, and after commitment that occurs by an intrinsic program of the cells, they may stimulate maintenance and maturation of progenitor cells.

Key words: GM-CSF, hematopoietic stem cell, stromal cell.

The many hematopoietic cell types are all derived from a single multipotent hematopoietic stem cell. Commitment of the stem cells to specific lineages is thought to occur by progressive loss of potential for alternative fates in a stochastic manner (*1*, *2*), but the molecular events underlying lineage commitment are not well understood. Cytokines affect the production of blood cells by interacting with their membrane receptors, which regulate the proliferation and survival of specific progenitor cells (*3*). In addition, cytokines may act as inducers of differentiation and lineage commitment. In fact, using IL-3–dependent multipotent murine stem cell lines (FDCP-mix), Just *et al.* (*4*) reported that single FDCP-mix cells infected with retroviral vectors expressing GM-CSF are induced to differentiate into granulocytes and macrophages, suggesting an instructive mechanism by GM-CSF receptor.

In hematopoietic organs such as bone marrow, self-renewal and commitment of multipotent hematopoietic stem cells are regulated in the hematopoietic inductive microenvironment created by the stromal cells. Studies on long-term bone marrow culture systems have shown that primitive stem cells can be maintained for a long time in culture on the stromal cell layers of bone marrow (*5*). We previously reported that one stromal cell line, TBR59, among many stromal cell lines established from bone marrow using temperature-sensitive (ts) T-antigen transgenic mice (*6*), can induce proliferation and commitment of differentiation to myeloid and B-lymphoid lineages of the sorted stem cells (*7*). In the present study, we examined whether commitment of the hematopoietic stem cells could be altered by cytokines using the sorted bone marrow stem cells from human GM-CSF receptor transgenic mice (*8*). The results indicated that hGM-CSF costimulates rapid proliferation of the stem cells with stromal cells, but does not affect lineage commitment.

MATERIALS AND METHODS

Transgenic Mice—To achieve ubiquitous expression of cDNAs of both α and β subunits of hGM-CSF receptor, each cDNAs was inserted into the pLG1 vector, which consisted of 1.2 kb of the 5′ flanking sequence from the mouse major histocompatibility complex (MHC) L-locus (H2-Ld) gene, 0.8 kb of the intron, and 0.5 kb of polyadenylation site from the rabbit β globin gene, and transgenic mice constitutively expressing high affinity hGM-CSF were generated as described (*8*). Their C57BL/6J background homozygous transgenic mice were mated with normal C57BL/6J females, and F1 were used for the isolation of the hematopoietic stem cells in bone marrow. The presence of transgene was examined by genomic polymerase chain reaction (PCR) by using

[1] This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan and by the Proposal-based New Industry Creative Type Technology R&D Promotion Program from the New Energy and Industrial Technology Development Organization (NEDO) of Japan.

[2] To whom correspondence should be addressed. Tel: +81-22-717-8483, Fax: +81-22-717-8488, E-mail: mobinata@idac.tohoku.ac.jp

0.5 μg of mouse tail tip DNA. The DNA was amplified with specific primers for the hGM-CSF α-subunit, 5′-TGGACTTTCAGTACCAGCTG-3′ and 5′-GTCTTTGATCTGTGGAACTG-3′ and β-subunit, 5′-AGGTCACCAAGGAACAATCCT-3′ and 5′-TTTGAAGAGCTGAATGAC-3′, using 0.25 U/ml AmpliTaq (Perkin Elmer, Branchburg, NJ). PCR was performed with 30 cycles of 94°C (30 s), 55°C (30 s), and 72°C (90 s). The PCR products were resolved on 2% agarose gel.

Hematopoietic Growth Factors—Recombinant hGM-CSF, recombinant murine IL-3, recombinant murine IL-6, and recombinant murine SCF were provided by Kirin (Tokyo).

Antibodies—Fluorescein isothiocyanate (FITC) conjugated anti-B220 (RA3-6B2), anti-TER19 (erythroid lineage marker), anti–Mac-1 (M1/70), anti–Gr-1 (RB6-8C5), anti-CD3 (145-2C11), and phycoerythrin (PE) conjugated anti–Sca-1 antibodies were purchased from Pharmingen (San Diego, CA) and used as lineage markers. Anti–c-Kit antibody (ACK2, generously supplied by Dr. S.-I. Nishikawa, Kyoto University) was coupled with biotin.

Cell Line and Culture Conditions—A mouse bone marrow stromal cell line (TBR59) established from temperature- sensitive SV40 T-antigen gene transgenic mice (*6*) was maintained in RITC 80-7 (Kyokuto Pharmaceutical, Tokyo) supplemented with 2% fetal bovine serum (FBS), 10 μg/ml transferrin, 10 ng/ml epidermal growth factor (generously supplied by Wakunaga, Tokyo), and 1 μg/ml insulin. Cultures were incubated in a humidified atmosphere of 5% CO_2 in air at 33°C.

Enrichment of Hematopoietic Stem Cells—A highly enriched stem cell fraction was obtained from bonemarrow of hGM-CSF transgenic mice by the modified method described previously (*13*). Briefly, bone marrow cells were incubated with a cocktail of FITC-conjugated monoclonal antibodies specific to lineage markers (B220, Mac-1, Gr-1, CD3, and TER119), then with PE-conjugated Sca-1 and biotinated ACK2 (anti–c-Kit monoclonal antibody), and finally with streptavidine-arophycocyanine (SA-APC) (Becton Dickinson, San Jose, CA) and propidium iodide. Lin^- $c\text{-}Kit^+$ $Sca1^+$ cells were sorted by use of FACStar Plus (Becton Dickinson) with an automated cell deposition unit.

In Vitro Methylcellulose Assay—Fractionated hematopoietic cells were resuspended in 0.5 ml of α-minimum essential medium (α-MEM) (Gibco BRL, Grand Island, NY) containing 30% FBS, 1% deionized bovine serum albumin, 1.2% methylcellulose, 100 μM 2-meraptoethanol (2-ME), SCF (100 ng/ml), IL-3 (200 U/ml), and IL-6 (20 ng/ml) in a 24-well plate (Falcon, Becton Dickinson). After 4 and 7 days of culture at 37°C, colonies consisting of more than 50 cells were counted in triplicate as colony-forming units (CFU-C), and colonies greater than 2 mm in diameter after 14 days were regarded as high proliferative potential colony-forming cells (HPP-CFC).

Cobblestone Formation of the Sorted Stem Cells on the Stromal Cells—For cocultivation of the sorted stem cells on stromal cell lines, confluent cell layers of TBR59 cells were formed in a six-well plate (Sumiron, Sumitomo Bakelite, Tokyo). The medium was then replaced with α-MEM supplemented with 10% FBS and 100 μM 2-ME, and Lin^- $c\text{-}Kit^+$ $Sca1^+$ cells were sorted onto each dish. The cultures were incubated at 37°C and 3/4 of the volume of the medium was changed every 3 days. Colonies formed beneath the stromal cell layers consisting of more than 10 cells were counted as cobblestones.

Flow Cytometrical Analysis—For immunofluorescence labeling, 1×10^6 cells were incubated with FITC-conjugated–anti-B220, anti–Gr-1, and anti–Mac-1 antibodies, PE-conjugated Sca-1, and biotin-conjugated ACK2 in bovine serum albumin dissolved in phosphate-buffered saline (BSA-PBS) for 30 min on ice, then incubated with SA-APC. The cells were washed three times with BSA-PBS and analyzed by use of FACStar Plus.

TABLE I. ***In vitro* methylcellulose assay of the sorted stem cells from hGM-CSF receptor transgenic mice.**

	CFU-C		HPP-CFC
	day 4	day 7	day14
No addition	2.0 ± 0.5	9.6 ± 1.4	2.6 ± 0.6
hGM-CSF (10 ng/ml)	9.0 ± 1.5	25.6 ± 1.3*	11.0 ± 1.5
hGM-CSF (100 ng/ml)	5.3 ± 0.8	21.3 ± 0.3	8.3 ± 0.8

One hundred sorted Lin^- $c\text{-}Kit^+$ $Sca\text{-}1^+$ cells were seeded in methylcellulose in the presence or absence of hGM-CSF. CFU-C were counted on day 4 and day 7 of culture, and HPP-CFC were counted on day 14. The values are the mean ± SD for three wells. (*$p < 0.0001$, one-way ANOVA).

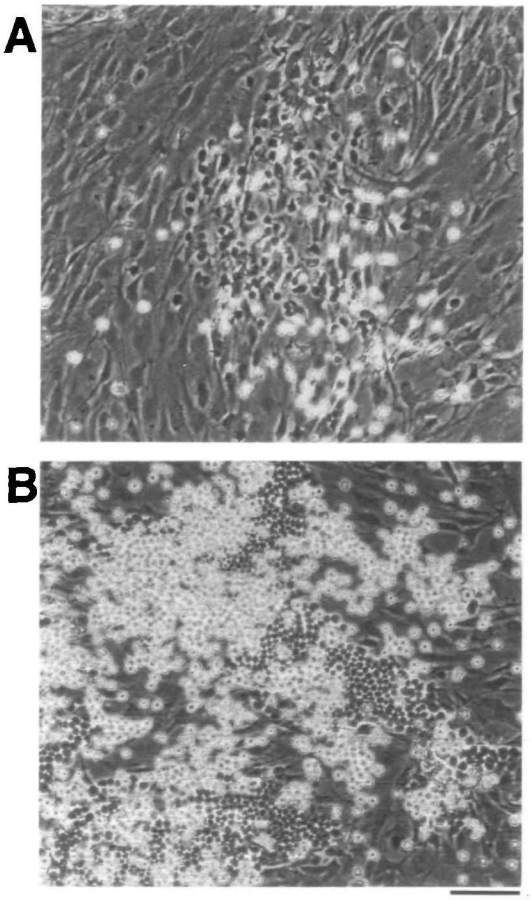

Fig. 1. **Cobblestone areas from sorted stem cells on the TBR59 stromal cell layers.** The sorted stem cells (100 cells/well) from transgenic mice were cocultured with a cell layer of TBR59 cells, and cobblestone areas in the absence (A) or presence of hGM-CSF (10 ng/ml) (B) were observed by a phase-contrast microscopy. Phase-dark colonies were formed underneath the layer after 4 days of coculture of the sorted stem cells. Bar indicates 100 μm.

RESULTS

Effect of hGM-CSF on Colony Formation in Semisolid Medium of Bone Marrow Hematopoietic Cells from hGM-CSF Receptor Transgenic Mice—To determine the colony-forming activity of the sorted stem cells from the hGM-CSF receptor transgenic mice, lineage negative (Lin^-), c-Kit^+ $Sca1^+$ cells were sorted by a cell sorter. The Lin^- c-Kit^+ $Sca1^+$ cells were used for *in vitro* methylcellulose assay (CFU-C and HPP-CFC) (Table I). Formation of both CFU-C and HPP-CFC by the sorted stem cells was greatly stimulated by addition of hGM-CSF. The number and size of colonies were significantly greater in the presence of hGM-CSF than in its absence, indicating that expansion of the sorted stem cells was strongly stimulated by signals mediated by the transduced human GM-CSF receptor. Morphologically, the cells in the colonies were mostly macrophages.

Effect of hGM-CSF on Cobblestone Formation on TBR59 Stromal Cells by the Sorted Hematopoietic Stem Cells from hGM-CSF Receptor Transgenic Mice—We previously demonstrated that an established bone marrow stromal cell line, TBR59, can induce cobblestone area formation committed to myelopoiesis and lymphopoiesis of the bone marrow hematopoietic stem cells sorted as Lin^- c-Kit^+ $Sca1^+$ cells (7). To examine the lineage commitment of the stem cells in the stroma-dependent culture, Lin^- c-Kit^+ $Sca1^+$ cells from the hGM-CSF receptor transgenic mice were cultured on the TBR59 stromal cell layers. The round cells in the hematopoietic cobblestone area were phase-dark, indicating that they lay underneath or were rigidly attached to TBR59 cells, and each cobblestone area formed distinct concentric circles, suggesting that they derived from a single cell, as previously reported (7). In the absence of hGM-CSF, small cobblestones were observed (Fig. 1A), but in the presence of hGM-CSF, larger cobblestones were formed (Fig. 1B) and their number was greatly increased by the addition of hGM-CSF (Table II.). The cells within the cobblestones formed in the presence of hGM-CSF on day 7 of coculture were analyzed by Giemsa staining and flow cyto-

TABLE II. **Cobblestone colony formation of the sorted stem cells from hGM-CSF receptor transgenic mice on TBR59 stromal cells.**

	Number of cobblestone colonies	
	Day 4	Day 7
No addition	2.6 ± 0.3	10.6 ± 0.6
hGM-CSF (10 ng/ml)	29.3 ± 1.4*	all '

One hundred sorted Lin^- c-Kit^+ $Sca\text{-}1^+$ cells were seeded on the TBR59 stromal cell layers in the presence or absence of hGM-CSF as described in "MATERIALS AND METHODS" and the cobblestones were counted on day 4 and day 7 of culture. The values are the mean ± SD for three wells. (*$p < 0.0001$, *t*-test. all ' indicates that the cobblestones covered the entire well.)

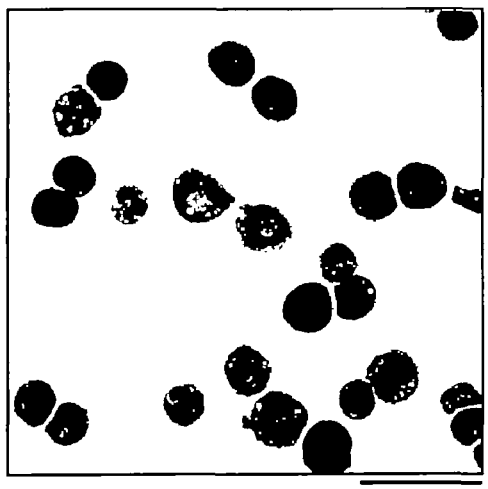

Fig. 2. **May-Gruenwald-Giemsa staining of the cells in the cobblestones formed in the presence of hGM-CSF after 7 days of culture. Bar indicates 50 μm.**

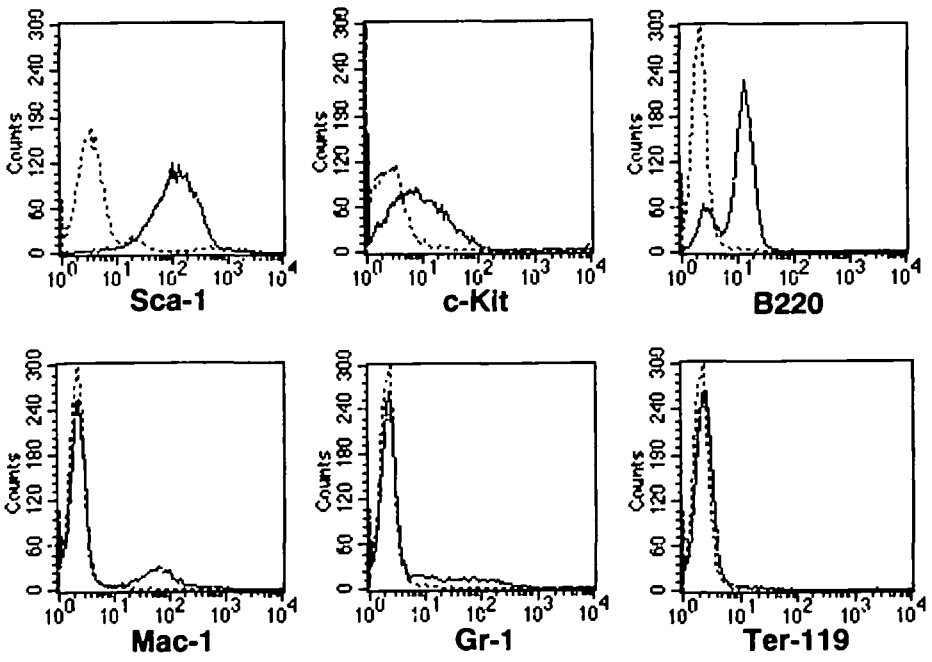

Fig. 3. **Lineage marker expression in the hematopoietic cells developed after 7 days of coculture of the sorted stem cells with TBR59 stromal cell layers.** The cells released into the culture medium were collected and stained with B220, Gr-1, Mac-1, TER119, c-Kit, or Sca-1 antibodies as described in "MATERIALS AND METHODS." Dotted lines indicate the negative control.

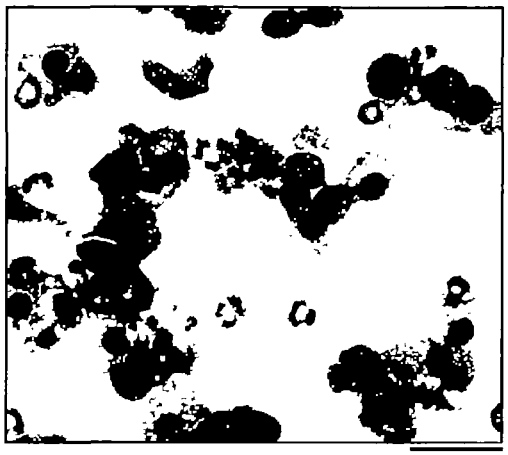

Fig. 4. **May-Gruenwald-Giemsa staining of the cells maintained on TBR59 stromal cells after four passages for 1 month in the presence of hGM-CSF.** Bar indicates 50 μm.

Fig. 5. **Mast cells maintained for 1 month in liquid culture in the presence of hGM-CSF.** Bar indicates 50 μm.

metry. Our previous work (*7*) showed that the hematopoietic cells within the cobblestones consisted mainly of granulocytes, macrophages, and B-lymphoid cells; and Giemsa staining of the cobblestone-forming cells of hGM-CSF receptor transgenic mice showed essentially similar patterns exhibiting multiple types of hematopoietic cells (Fig. 2). Flow cytometrical analysis (Fig. 3) showed that over 95% of the cells were Lin^+ cells, and thus a dominant portion of the stem cell fraction sorted as Lin^- c-Kit^+ $Sca1^+$ cells became differentiated in this culture. Unexpectedly, the majority of the cells (80%) were lymphoid cells ($B220^+$), while macrophages ($Mac\text{-}1^+$) (17%) and granulocytes ($Gr\text{-}1^+$) (14%) were in low abundance. This contrasted with our previous result (*7*) using the sorted stem cells from the non-transgenic mice, where 70–80% of the cells were granulocytes and 30–20% of cells were lymphoid cells. In both cases, erythroid cells ($Ter119^+$) were not detected.

Prolonged Culture of the Sorted Stem Cells—In addition to the transient effect of hGM-CSF on the sorted stem cells, its effect was examined in the prolonged culture of these cells to determine whether hGM-CSF–dependent and stroma-dependent primitive hematopoietic cell lines could be generated. In the stroma-supported culture, the cobblestone-forming cells were repeatedly transferred to fresh stromal cell layers in the presence of hGM-CSF. Although we failed to maintain these cells in the previous work using stem cells from non-transgenic mice, the cells generated from the hGM-CSF receptor transgenic mice could be maintained longer in the presence of hGM-CSF on the stromal cell layers. Cell proliferation terminated after four transfers, but interestingly, the cells remaining in the final stage (Fig. 4) were morphologically distinct from those in the day 7 culture (Fig. 2) and the majority of the cells were macrophages or granulocytes.

Similarly, we examined whether the cells in the colonies of semisolid assay could be maintained in a liquid culture in the presence of hGM-CSF. Cells were maintained over 1 month by repeated passages in the presence of hGM-CSF, and at that time most showed the morphology typical of mast cells (Fig. 5). Thus, under different conditions, hGM-CSF supported maintenance of different progenitor cells derived from the sorted stem cells.

DISCUSSION

The relation between receptor expression and differentiation of hematopoietic cells was determined by generating transgenic mice that constitutively expressed hGM-CSF receptor at almost all stages of hematopoietic cell development. hGM-CSF was shown to support various types of colonies, including granulocyte-macrophage, mast cell, megakaryocyte, blast cell, and mixed hematopoietic colonies, whereas mouse GM-CSF supported only granulocyte-macrophage colonies (*8*). In addition, *in vivo* administration of hGM-CSF to transgenic mice resulted in a dose-dependent increase in reticulocytes and white blood cells in the peripheral blood, and hGM-CSF generated erythrocyte colonies in the absence of erythropoietin (*9*). These results indicate that hGM-CSF receptor–mediated signals can support the growth of cells of all hematopoietic cell lineages if this receptor is present on the cell surface. The transgenic mouse models of the receptors for erythropoietin or IL-5 gave essentially similar results (*10–13*). These findings favor the stochastic model in which the differentiation of hematopoietic progenitor cells is determined by an intrinsic cell program in which cytokines simply select cells that express the appropriate receptor, rather than the instruction model in which it is determined by exogenous cytokine stimulation.

Contrary to the above results, GM-CSF was found to induce the transfected cells to differentiate into granulocytes and macrophages when GM-CSF receptor gene was introduced into single multipotent FDCP-mix cells by retroviral vectors, and this suggested an instructive mechanism of the GM-CSF receptor (*4*).

We wanted to learn how the cytokine receptor affects the differentiation program of the stem cells by the direct action of hGM-CSF on the sorted stem cells from bone marrow of the hGM-CSF receptor transgenic mice using a newly established coculture system (*7*); in this system the established stromal cell line, TBR59, can induce a finite number of divisions committed to myelopoiesis and lymphopoiesis of the bone marrow hematopoietic stem cells sorted as Lin^- c-Kit^+ $Sca\text{-}1^+$ cells. In this coculture, the commitment to differentiation of the stem cells toward myeloid and lymphoid cells was observed in the absence of

exogenously added soluble factors, and antibodies for c-Kit or very late activation antigen-4 (VLA-4) were shown to inhibit cobblestone formation. Thus, at least in part, signals mediated through c-Kit, a receptor for SCF (stem cell factor), are necessary. We asked whether the fates of the hGM-CSF receptor-expressing stem cells could be switched toward granulocyte/macrophage lineages by hGM-CSF. hGM-CSF strongly stimulated the cobblestone formation, in which both granulocyte/macrophage and B-lymphoid lineages were generated from the sorted stem cells. Thus, hGM-CSF may not act on the stem cells to determine their differentiation to granulocyte/macrophage lineage, but may act only as costimulator to the signals provided by the stromal cells. In this study, therefore we found the stochastic model more favorable.

When the cells were cultured for a long period in the presence of hGM-CSF on the stromal cell layers, the cobblestone formation was gradually reduced and the majority of remaining cells became granulocytes and macrophages. This suggested that at the earlier phase of culture, the signals from the stromal cells may act dominantly on the commitment of the stem cells, and hGM-CSF may provide only growth signals costimulatory with the signal provided by the stromal cells. However, once the progenitor cells were generated, the signals from the stromal cells, though still necessary, became weaker, and hGM-CSF may act more dominantly than the stromal signals on the progenitor cells. hGM-CSF may stimulate growth, and/or induce maturation of the myeloid progenitor cells. This mechanism is supported by our previous reports that rapid expansion and differentiation of myeloid progenitor cells to granulocytes or macrophages were stimulated by particular stromal cell clones in the absence of cytokines; thus, the myeloid progenitors may have a stroma-dependent phase in their development (*14*). We also reported that THS119, a stroma-dependent primitive hematopoietic cell line, acquired IL-3 or IL-7 dependence only after prolonged culture with the stromal cells in the presence of these cytokines (*15*). In addition, we recently generated a stroma-dependent myeloid progenitor cell clone that can differentiate to granulocytes and macrophages and can be costimulated by GM-CSF and IL-3 with the stromal cells (Okubo *et al.*, in preparation).

In contrast to the coculture, only mast cells were maintained by hGM-CSF for a long period in suspension culture of the cells from the semisolid assay, which had contained macrophages and, possibly, their progenitors. Granulocytes or macrophages may not be maintained by hGM-CSF even when its receptor is abundantly expressed, but may use a similar signaling pathway to IL-3 in mast cells, since mast cells are known to be maintained easily for a long period in suspension culture in the presence of IL-3, or it may costimulate with other serum factor(s).

We could not detect TER119-positive erythroid cells from the hGM-CSF receptor–transduced stem cells, and this is consistent with the previous observation that no erythroid cells were generated from the sorted stem cells even with the addition of erythropoietin to our coculture system (*7*). However, it contrasted with the observations that *in vivo* administration of hGM-CSF to transgenic mice resulted in an increase in reticulocytes in the peripheral blood, and that hGM-CSF generated erythrocyte colonies in the absence of erythropoietin (*8*). hGM-CSF may act with other signals to costimulate the erythroid progenitor cells of the transgenic mice, similar to the gp130 signaling association with c-kit activation in human erythropoiesis *in vivo* (*16*).

These results consistently suggest that cytokines such as GM-CSF may costimulate primitive hematopoietic cells at their stroma-dependent phase before lineage commitment, and after commitment they may stimulate growth and maturation of the progenitor cells.

REFERENCES

1. Ogawa, M., Porter, P.N., and Nakahata, T. (1983) Renewal and commitment to differentiation of hemopoietic stem cells (an interpretive review). *Blood* **61**, 823–829
2. Ogawa, M. (1990) Differentiation and proliferation of hematopoietic stem cells. *Blood* **81**, 2844–2853
3. Arai, K., Lee, F., Miyajima, A., Miyatake, S., Arai, N., and Yokota, T. (1990) Cytokines: Coordinators of immune and inflammatory responses. *Annu. Rev. Biochem.* **59**, 783–836
4. Just, U., Stocking, C., Spooncer, E., Dexter, T.M., and Ostertag, W. (1991) Expression of the GM-CSF gene after retroviral transfer in hematopoietic stem cell lines induces synchronous granulocyte-macrophage differentiation. *Cell* **22**, 1163–1173
5. Dexter, T.M., Allen, T.D., and Lajtha, L.G. (1977) Conditions controlling the proliferation of hematopoietic stem cells in vitro. *J. Cell Physiol.* **91**, 335–344
6. Kameoka, J., Yanai, N., and Obinata, M. (1995) Bone marrow stromal cells selectively stimulate the rapid expansion of the lineage-restricted myeloid progenitors. *J. Cell Physiol.* **164**, 55–64
7. Okuyama, R., Koguma, M., Yanai, N., and Obinata, M. (1995) Bone marrow stromal cells induce myeloid and lymphoid development of the sorted hematopoietic stem cells in vitro. *Blood* **86**, 2590–2597
8. Nishijima, I., Nakahata, T., Hirabayashi, Y., Inoue, T., Kurata, H., Miyajima, A., Hayashi, N., Iwakura, Y., Arai, K., and Yokota, T. (1995) A human GM-CSF receptor expressed in transgenic mice stimulates proliferation and differentiation of hemopoietic progenitors to all lineages in response to human GM-CSF. *Mol. Biol. Cell* **6**, 497–508
9. Nishijima, I., Nakahata, S., Watanabe, S., Tsuji, K., Tanaka, Y., Hirabayashi, T., Inoue, T., and Arai, K. (1997) Hematopoietic and lymphopoietic responses in human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor transgenic mice injected with human GM-CSF. *Blood* **90**, 1031–1038
10. Pharr, P.N., Hankins, D., Hofbauer, A., Lodish, H.F., and Longmore, G.D. (1993) Expression of a constitutively active erythropoietin receptor in primary hemopoietic progenitors abrogates erythropoietin dependence and enhances erythroid colony-forming unit, erythroid burst forming unit, and granulocyte/macrophage progenitor growth. *Proc. Natl. Acad. Sci. USA* **90**, 938–942
11. Dubart, A., Feger, F., Lacout, C., Goncalves, F., Vainchenker, W., and Dumenil, D. (1994) Murine pluripotent hematopoietic progenitors constitutively expressing a normal erythropoietin receptor proliferate in response to erythropoietin without preferential erythroid cell differentiation. *Mol. Cell. Biol.* **14**, 4834–4842
12. Pharr, P.N., Ogawa, M., Hofbauer, A., and Longmore, G.D. (1994) Expression of an activated erythropoietin or a colony-stimulating factor 1 receptor by pluripotent progenitors enhances colony formation but does not induce differentiation. *Proc. Natl. Acad. Sci. USA* **91**, 7482–7486
13. Takagi, M., Hara, T., Ichihara, M., Takatsu, K., and Miyajima, A. (1995) Multi-colony stimulating activity of interleukin 5 (IL-5) on hematopoietic progenitors from transgenic mice that express IL-5 receptor alpha subunit constitutively. *J. Exp. Med.* **181**, 889–899
14. Iguchi, A., Okuyama, R., Koguma, M., Yanai, N., and Obinata, M. (1997) Bone marrow stromal cells induce granulocytic development in the absence of G-CSF. *Cell Struct. Funct.* **22**, 357–364

15. Yanai, N., Matsui, N., Matsuda, K.-I., Furusawa, T., Okubo, T., Nakazawa, T., Ishibashi, K., Nawa, K., and Obinata, M. (1999) A novel stromal cell-dependent hematopoietic cell line established from temperature-sensitive SV40 T-antigen transgenic mice. *Exp. Hematol.* **27**, 1087–1096
16. Sui, X., Tsuji, K., Tajima, S., Tanaka, R., Muraoka, K., Ebihara, Y., Ikebuchi, K., Yasukawa, K., Taga, T., Kishimoto, T., and Nakahata, T. (1996) Erythropoietin-independent erythrocyte production: signals through gp130 and c-kit dramatically promote erythropoiesis from human $CD34^+$ cells. *J. Exp. Med.* **183**, 837–845